

39. (NEW) A nucleic acid comprising a nucleic acid sequence which encodes the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A and is capable of being expressed in an organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line.

40. (NEW) The nucleic acid of claim 39, wherein the gram negative bacteria is *Escherichia coli*.

41. (NEW) The nucleic acid of claim 39, wherein the yeast is *Pichia pastoris*.

42. (NEW) The nucleic acid of claim 39, wherein said nucleic acid comprises the nucleic acid sequence of SEQ ID No. 1, SEQ ID No. 3, SEQ ID No. 5, or combinations thereof. N.M.

43. (NEW) A nucleic acid comprising a sequence which encodes a polypeptide having the sequence of SEQ ID No. 2, SEQ ID No. 4, SEQ ID No. 6, or combinations thereof. N.M.

44. (NEW) The nucleic acid of claim 39, wherein said nucleic acid is a synthetic nucleic acid.

45. (NEW) The nucleic acid of claim 39, wherein said nucleic acid is operably linked to expression control sequences.

46. (NEW) The nucleic acid of claim 39, wherein said expression control sequences comprise a promoter.

47. (NEW) The nucleic acid of claim 39, wherein said expression control sequences comprise an enhancer.

Sub E3
48. (NEW) A method of preparing a polypeptide comprising the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A, said method comprising transfecting an organism with the nucleic acid of claim 39, culturing the transfected organism under conditions wherein the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A is expressed, wherein the organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line..

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49. (NEW) The method of claim 48, further comprising recovering insoluble protein from said transfected organism.

50. (NEW) The method of claim 48, wherein said organism is *Escherichia coli*.

51. (NEW) The method of claim 48, wherein said organism is *Pichia pastoris*.

52. (NEW) An immunogenic composition comprising the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A.

Sub E4
53. (NEW) A method of preparing the immunogenic composition of claim 52, said method comprising culturing a recombinant host organism transfected with an expression vector encoding, in an expressible form, the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A.

54. (NEW) A method of preparing the immunogenic composition of claim 52 comprising culturing a recombinant organism capable of expressing the carboxy-terminal portion of the heavy chain of botulinum neurotoxin and recovering an insoluble protein fraction from the recombinant organism.

55. (New) The nucleic acid of claim 39, wherein the A+T content is less than about 70% of the total base composition.

56. (New) The nucleic acid of claim 55, wherein the A+T content is less than about 60% of the total base composition.

57. (NEW) A nucleic acid comprising a nucleic acid sequence which encodes the amino-terminal portion of the heavy chain of botulinum neurotoxin serotype A and is capable of being expressed in an organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line.

58. (NEW) The nucleic acid of claim 57, wherein the gram negative bacteria is *Escherichia coli*.

59. (NEW) The nucleic acid of claim 57, wherein the yeast is *Pichia pastoris*.

60. (NEW) The nucleic acid of claim 57, wherein said nucleic acid comprises the nucleic acid sequence of SEQ ID No. 19.

61. (NEW) A nucleic acid comprising a sequence which encodes a polypeptide having the sequence of SEQ ID No. 20.

62. (NEW) The nucleic acid of claim 57, wherein said nucleic acid is a synthetic nucleic acid.
63. (NEW) The nucleic acid of claim 57, wherein said nucleic acid is operably linked to expression control sequences.
64. (NEW) The nucleic acid of claim 57, wherein said expression control sequences comprise a promoter.
65. (NEW) The nucleic acid of claim 57, wherein said expression control sequences comprise an enhancer.
66. (New) The nucleic acid of claim 57, wherein the A+T content is less than about 70% of the total base composition.
67. (New) The nucleic acid of claim 66, wherein the A+T content is less than about 60% of the total base composition.
68. (NEW) A method of preparing a polypeptide comprising the amino-terminal portion of the heavy chain of botulinum neurotoxin serotype A, said method comprising
transfecting an organism with the nucleic acid of claim 57,
culturing the transfected organism under conditions wherein the amino-terminal portion of the heavy chain of botulinum neurotoxin serotype A is expressed, wherein the organism selected from the group consisting of a gram negative bacteria, a yeast, and a mammalian cell line..

69. (NEW) The method of claim 68, further comprising recovering insoluble protein from said transfected organism.

70. (NEW) The method of claim 68, wherein said organism is *Escherichia coli*.

71. (NEW) The method of claim 68, wherein said organism is *Pichia pastoris*.

72. (NEW) An immunogenic composition comprising the amino-terminal portion of the heavy chain of botulinum neurotoxin serotype A.

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73. (NEW) A method of preparing the immunogenic composition of claim 72, said method comprising culturing a recombinant host organism transfected with an expression vector encoding, in an expressible form, the amino-terminal portion of the heavy chain of botulinum neurotoxin serotype A.

74. (NEW) A method of preparing the immunogenic composition of claim 72 comprising culturing a recombinant organism capable of expressing the amino-terminal portion of the heavy chain of botulinum neurotoxin and recovering an insoluble protein fraction from the recombinant organism.

75. (NEW) An immunogenic composition comprising at least a portion of the heavy chain of botulinum neurotoxin serotype A.

76. (NEW) The immunogenic composition of claim 75, wherein said portion of the heavy chain of botulinum neurotoxin serotype A elicits an ELISA response to botulinum

neurotoxin serotype A in an animal, said ELISA response being detectable upon about 100-fold dilution of serum from said animal.

77. (NEW) The immunogenic composition of claim 75, wherein said portion of the heavy chain of botulinum neurotoxin serotype A comprises at least one epitope of the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A or one epitope of the amino-terminal portion of the heavy chain of botulinum neurotoxin serotype A, wherein said epitope is capable of eliciting protective immunity toward botulinum neurotoxin serotype A.

B₃ 78. (NEW) The immunogenic composition of claim 77, wherein said immunogenic composition elicits an ELISA response to botulinum neurotoxin serotype A in an animal, said ELISA response being detectable upon about 100-fold dilution of serum from said animal.

79. (NEW) The immunogenic composition of claim 75, wherein said composition is endotoxin free.

80. (NEW) A nucleic acid encoding a protein comprising at least one epitope of the heavy chain of botulinum neurotoxin serotype A.

81. (NEW) The nucleic acid of claim 80, wherein said protein is a fusion protein further comprising a non-toxic polypeptide sequence.

82. (NEW) A recombinant host cell comprising the nucleic acid of claim 39, 57, or both.

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can not
depend
on
claim
39, 57, or both